THE EFFECT OF HYDROCORTISONE ON THE ACCUMULATION OF AMYLASE IN EMBRYONIC CHICK PANCREAS

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Received 1 February 1969

1. Introduction

During the late stages of development of the chick embryo amylase and other exportable enzymes accumulate rapidly in the pancreas from about 17 days of development until 2 days after hatching (developmental age 22 days) [1–3]. Although this phase of rapid accumulation of digestive enzymes is dramatic and apparently plays a vital role in pancreatic development, it is clearly the consequence of the maturation of an already highly differentiated organ [4]. Immediately prior to the maturation phase (from 12 to 16 days of development) amylase specific activity remains constant. These observations implied that there must be an early phase of differentiation before 12 days of development, which should be characterized by the rapid accumulation of amylase. The present study shows that there is indeed an early phase of differentiation which starts before 6 days of development and that during this phase, which ends at 12 days of development, the specific activity of amylase increases by more than 2 orders of magnitude. Pancreases from 7-day embryos cultured *in vitro* underwent a limited degree of differentiation as indicated by the accumulation of amylase. The rate of accumulation of amylase in pancreas cultured *in vitro* could be greatly increased by adding hydrocortisone to the medium even after accumulation in the absence of the hormone had stopped.

2. Materials and methods

Embryos were from White Leghorn x New Hampshire eggs incubated at 38°.

The organ culture technique was based on that of Wolff and Haffen [5]. In addition to the normal constituents the medium contained 23% horse serum and 50 μg/ml of crystalline soybean trypsin inhibitor (Worthington). Each culture dish contained 3 whole pancreases from 7-day embryos which were placed on a dialysis membrane resting on 2 ml of medium. A solution of hydrocortisone in ethanol was added where indicated and an equivalent amount of ethanol (not exceeding 0.25%) was added to the controls. Data presented are the mean for cultures set up in triplicate. At the end of the incubation the membrane with the explants was rinsed with Krebs Ringer phosphate and the explants were homogenized with 0.03 M phosphate buffer pH 6.9 containing 7 mM NaCl.

In the *in vitro* experiments amylase was determined by the following micro-modification of the Bernfeld method [6]. The volume of the reaction mixture was reduced to one-quarter and the incubation was carried out for 30 min at 37°. A unit of enzyme is defined as the amount which under these conditions releases reducing groups equivalent to 0.5 mg of maltose hydrate. Protein was determined by the method of Lowry et al. [7].

3. Results

3.1. *Changes in the specific activity of amylase during the differentiation of pancreas in vivo*

Already at 6 days of embryonic development, which the earliest stage at which we have been able to isolate the pancreatic primordium, the specific activity of amylase in the pancreas is more than ten times higher than that in other tissues (fig. 1). Three phases

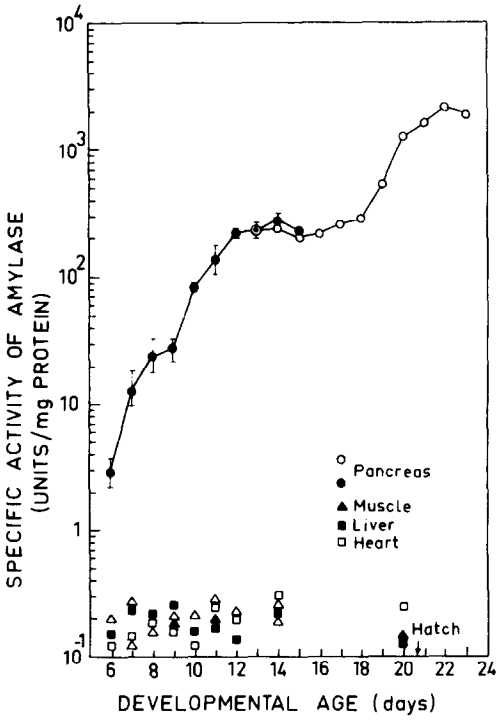

Fig. 1. Developmental changes in specific activity of amylase in pancreas and other tissues of the chick embryo. Homogenates of 5–20 pancreases were used for each determination. Data for pancreas (closed circles) are the mean of estimations on 3–5 homogenates; vertical bar shows range of individual values. Data for pancreas (open circles) were taken from Marchaim and Kulka [2]. Points for extrapancreatic tissues represent the mean of at least two determinations. Amylase was determined by the amylolytic method [4], modified by using 5% trichloroacetic acid to precipitate protein before measurement of residual starch. Specific activity of amylase is expressed as micro-Bernfeld units/mg protein for comparison with *in vitro* experiments.

of accumulation of amylase are clearly discernible. Between 6 to 12 days of incubation the specific activity of amylase increases by about two orders of magnitude. From 13 to 16 days there is no change in the specific activity of amylase while from 17 to 22 days there is a sharp increase of one order of magnitude which has been described previously [1]. In extrapancreatic tissues the specific activity of amylase remains at a constant low level throughout development.

3.2. *Accumulation of amylase in organ culture*

In pancreases cultured for 6 days *in vitro* on medium not supplemented with hormones the specific activity of amylase increased 3 to 4-fold in most experiments (table 1). In some experiments considerably greater increases (up to 10-fold) were observed. As the total protein per pancreas did not increase dur-

Table 1
Accumulation of amylase in pancreas cultured *in vitro* without added hormones.

Duration of culture (days)	Protein per pancreas (μg)	Amylase per pancreas (units)	Specific activity of amylase (units/mg protein)
0	25	0.6	24
3	16 (14–20)	1.2 (0.7–1.6)	76 (47–118)
6	21 (17–25)	1.9 (1.5–2.3)	91 (90–92)

Numbers in parentheses show the range of values for individual cultures.

Table 2
Effect of hydrocortisone on the accumulation of amylase in pancreas cultured *in vitro*.

Duration of culture (days)	Protein per pancreas (μg)		Amylase per pancreas (units)		Specific activity of amylase (units/mg protein)	
	Minus HC *	Plus HC	Minus HC	Plus HC	Minus HC	Plus HC
0	25	–	0.85	–	34	–
3	21	18	1.0	4.5	47	250
6	33	29	2.6	12.7	81	448

* HC = hydrocortisone.
Hydrocortisone was added at the beginning of the experiment to give a final concentration of 5 μg/ml.

ing culture, the rise in the specific activity of amylase was due to an increase in total amylase (table 1).

3.3. *The effect of hydrocortisone on the accumulation of amylase in organ culture*

Addition of hydrocortisone to the culture medium raised the specific activity of amylase to about 5 times that of the control (table 2). As in the case of the controls there was little or no change in the total protein per pancreas and the increase in the specific activity of amylase was due to a rise in total amylase.

The level of amylase in pancreas explants could be increased by the addition of hydrocortisone after different periods of incubation even when the specific activity of the control cultures had reached a maximum. The later the hydrocortisone was added to the culture the sharper was the rise in the specific activity of amylase (fig. 2).

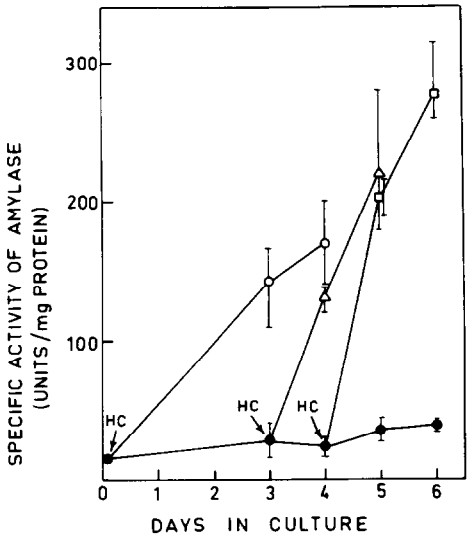

Fig. 2. Effect of adding hydrocortisone after different times of culture on accumulation of amylase in pancreas explants. The final concentration of hydrocortisone was 1 μg/ml. Arrows indicate times of addition of hydrocortisone (HC). ● control; ○, △, □ plus hydrocortisone.

4. Discussion

The present study confirms the existence of an early phase of differentiation of the pancreas, as shown by the accumulation of amylase, which starts before 6 days of development. By 12 days of development the specific activity of amylase reaches a level 3 orders of magnitude higher than the basal level found in tissues other than pancreas. Thus, on the basis of the relative increase of amylase specific activity a large measure of differentiation occurs during the early phase, while a much smaller relative increase in amylase specific activity occurs during the maturation phase. It should be remembered, however, that in terms of total amounts relatively little amylase accumulates during the first phase (about 1.5% of the total protein) whereas during the final (maturation) phase amylase becomes a major cell constituent comprising 12% of the total protein [1]. It seems, therefore, that during the first phase of differentiation the pancreatic primordium acquires the biochemical properties of pancreas (namely the capacity to synthesize characteristic digestive enzymes) while during the maturation phase the production of specific proteins is amplified to amounts suitable to the secretory role of the gland.

The organ culture experiments were performed in an attempt to clarify the nature of the two step increase of amylase specific activity *in vivo*. In most organ culture experiments with unsupplemented medium the specific activity of amylase reached a value equivalent to that of pancreas of 10-day embryos, but in several experiments it reached values corresponding with those of pancreas from 11 to 12-day embryos (i.e. the end of the first phase of amylase accumulation *in vivo*). Changes in total amylase and total protein, unlike changes in specific activity, were very much smaller *in vitro* than *in vivo*.

When hydrocortisone was added to cultures at zero time, specific activities of amylase after 6 days of incubation reached levels of pancreas of 19-day embryo *in vivo* (i.e. levels corresponding to the third step of differentiation). The effect of hydrocortisone appears to be superimposed on the differentiation without added hormones. This is indicated by the fact that the steepness of the increase of amylase specific activity is greater the longer the differentiation has proceeded before addition of hydrocortisone. A possible explanation of the phenomenon is that a primary event of differentiation (namely, an increase in the capacity to synthesize amylase) which occurs in the absence of the hormone, is amplified on addition of the hormone. Hydrocortisone has previously been shown to induce the synthesis of specific proteins in differentiating [8–10] and non-differentiating animal

cells [11] presumably by stimulating the production of specific messenger RNA's. Whether hydrocortisone acts on the chick pancreas by a similar mechanism remains to be determined. It is possible that the differentiation *in vitro* without added hormone corresponds with the primary phase *in vivo* while the hydrocortisone-stimulated differentiation corresponds with the maturation phase. This could be connected with the recent observation that the differentiation of the retina, which *in vivo* occurs at the same time as the maturation phase of the pancreas, is also induced *in vitro* by hydrocortisone [8,9]. Cortical steroids may thus be required for the final phase of differentiation of various organs of the chick embryo.

Acknowledgements

We are indebted to Professor Et.Wolff for his valuable advice on organ culture techniques. Mrs. Rachel Ampel gave excellent technical assistance. This work was supported by grant No. AM-09573 from the National Institutes of Health, U.S. Public Health Service.

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